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THE PATHOGENICITY OF B. ABORTUS AND B. MELITENSIS FOR MONKEYS

STUDIES ON THE GENUS BRUCELLA NOV. GEN. III.

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Recent observations made by A. C. Evans,[1] by Meyer and Fleischner, Shaw and Feusier[2] and by Zeller[3] have proven that B. abortus and B. melitensis are morphologically, biochemically and serologically closely related. In order to demonstrate conclusively the relationship of the two bacteria, it becomes necessary to conduct infection experiments on animals. In several series on guinea-pigs, it has been demonstrated that the lesions produced in the spleen, the lymph nodes, etc., following an intratesticular inoculation of certain B. melitensis strains, are indistinguishable from those produced by B. abortus. As the majority of the experiments by early observers on B. melitensis has been carried out on the monkey, an animal which, as originally shown by Bruce[4] and afterward by Hughes,[5] can be easily and certainly infected, it has seemed advisable to make similar experiments with B. abortus. The various reports of the Commission for the Investigation of Mediterranean Fever record numerous experiments dealing with infections produced in monkeys by subcutaneous, intramuscular and intravenous inoculations. Even the feeding of a single sample of infected goat's milk to 28 monkeys caused in 26 of them a disease which was very similar to that seen in man. It is stated that, as a rule, a B. melitensis infection of a monkey can be judged by the animal's temperature, which may be intermittent or remittent in character, by the appearance of specific agglutinins in the blood serum, and by slow, but definite loss in body weight.

As soon as the close relationship of B. abortus to B. melintensis had been established, it was manifestly important to determine experi-

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[1] Jour. Infect. Dis., 1918, 22, p. 580.

[2] Proc. Soc. Exper. Biol and Med., 1919, 16, p. 151; Jour. Infect. Dis., 1920, 27, pp. 173 and 185.

[3] Berl. tierarztlich. Wchnschr., 1920, 36, p. 345.

[4] Ann. de l'Instit. Pasteur, 1893, 7, p. 289.

[5] Lancet, 1892, 2, p. 1265.

mentally the following question: Can B. abortus produce in the monkey a similar syndrome as B. melitensis? Careful review of the work that had been done with B. abortus showed that only one investigator used monkeys. Marshal Fabyean,[6] in his studies on the persistence of this organism in the tissues of inoculated animals, infected two monkeys and was able to recover B. abortus 12 and 13 weeks, respectively, after the inoculation. He did not state the method used in inoculating the animals and gave no additional observations as to the results.

Aside from reproducing an undulant fever-like disease by direct inoculation of B. abortus, it was obviously important to determine carefully the possibility of causing an infection by feeding either cultures or goats milk containing the bacteria in small or large numbers. These experiments led to a consideration of the so-called "virulence" of B. abortus and resulted in a number of similar experiments with strains of different origin. It seemed also possible that more definite knowledge might be obtained concerning the pathogenicity of B. abortus for children and adults, if a study of feeding infections in monkeys were undertaken. The experiments reported here, therefore, were planned to supply a number of facts, which would assist in answering the question in a somewhat more intelligent manner than had hitherto been possible.

In order to be familiar with the temperature reactions, and the symptoms produced in monkeys by the organisms of malta fever, a number of infection experiments were made with certain interesting strains of B. melitensis. These cultures had been found to be pathogenic for guinea-pigs, and quite frequently they could not be differentiated from those of true abortion bacilli.

METHODS

Most of the experiments were made on young monkeys and if possible on one species, namely, Pithecus syrichta, imported from India. All were fresh stock and were brought directly to the laboratory on their arrival by steamer in San Francisco. On two occasions two old stock animals, a Macacus cynomolgus and a Cercopithecus philippinensis were also used. The animals were kept in single cages and special care was taken to avoid cross infection by contaminated food, urine, etc. This precaution was particularly necessary in connection with the B. melitensis experiments.

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[6] Jour. Med. Res., 1913, 28, p. 82.

The organisms used for the tests were grown on glycerol peptic digest agar slants (PH 7.0) at 37 C. for 2-7 days. The monkeys were fed by either smearing the bacterial growth on carrots or by suspending the cultural growth in 50-100 c c of milk. The latter was offered to the starved animals in small pannikins. As a rule, the monkeys drank the milk without wasting more than 20-30 c c. Blood stream inoculations were made by way of the jugular or saphenous vein.

The methods of study after inoculation or feeding consisted in the observation and record of clinical symptoms, the taking of morning temperature (rectal), repeated agglutination tests with the blood serum, and hemoglobin determinations. Formalinized suspensions of B. abortus and B. melitensis as specified in previous papers were used as antigens for the agglutination tests. Necropsies with bacteriologic examinations were made on all animals that died or were killed. Cultures of the tissues were made on blood agar plates, glycerol peptic digest agar slants and glycerol veal infusion broth. The strains isolated were identified by agglutination and absorption tests, as detailed in paper II of this series.

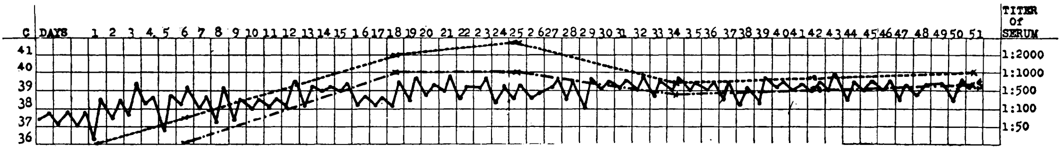

Chart 1 (Monkey 2).—Intravenous inoculation of B. abortus.

EXPERIMENTAL DATA

Infections Produced by the Intravenous Inoculations of B. abortus.—Three normal monkeys were injected intravenously with typical abortion bacilli in amounts ranging from 2-16 billion of a glycerol peptic digest agar culture. The following protocols detail the various observations.

Exper. 1.—*Monkey* 2.—Pithecus syrichta, female, weight 1,875 gm. March 18, to April 3, 1919, well and active; serum repeatedly examined for B. abortus and B. melitensis agglutinins with negative results. April 3, 3:00 p. m., inoculated intravenously in left jugular vein ⅕ slant of a 48-hour old peptic digest agar slant—2 billion of B. abortus 80 (origin of culture—certified milk—guinea-pig passage, 2½ years old); bled from the right ear 15 minutes after injection.

Blood smeared on agar slant gave profuse growth of B. abortus. The temperature during the period of observation of 51 days was always above 39 C. (chart 1).

April 24, the animal developed diarrhea which lasted for 2 days. Blood cultures on the sixth and eighteenth day remained sterile. The blood gave the following agglutination reactions:

April 9, or 6 days after injection—B. abortus 80=1:80; B. melitensis 11=0.

April 21, or 18 days after injection—B. abortus 80=1:2000; B. melitensis 11=1:1000.

April 28, or 25 days after injection—B. abortus 80=1:4000+; B. melitensis 11=1:1000.

May 7, or 34 days after injection—B. abortus 80=1:600 (inhibition zone 1:200); B. melitensis 11=1:400.

May 24, 1919, or 51 days after injection—B. abortus 80=1:1000 + + +; B. melitensis 11=1:600 + + +.

Skin tests conducted May 7 and 22 with aborto-and melitensis protein were negative. May 24, the animal was chloroformed and cultures carefully made, 51 days after the intravenous infection.

Postmortem Examination.—The cadavar was slightly emaciated and weighed 1,680 gm., a loss of 200 gm. The spleen was normal in size and light in color, and the lymph nodes were distinctly enlarged and rather firm. The liver, kidneys, and lungs appeared normal. A small superficial ulceration was found on the mucous membrane of the rectum. This lesion was probably caused by the careless insertion of the thermometer. Numerous trichocephalus worms were noted in the appendix and colon. Cultures were made from the heart blood, spleen, liver, kidneys, bile, mesenteric, mediastinal, femoral and axillary lymph nodes, bone marrow, lungs and urine. The agar slants inoculated with liver, spleen, right and left kidneys and femoral lymph nodes were densely crowded with colonies of B. abortus.

Exper. 2.—Monkey 11.—Pithecus syrichta (Linn.), male, weight 2180 gm. Agglutination tests repeatedly were negative to B. abortus and B. melitensis. Aug. 6, 1919, at 2:00 p. m., the animal was inoculated intravenously with ½ slant or 16 billion B. abortus No. 14 (isolated from a fetal membrane of a cow in 1917). The monkey never suffered from fever and was always active. The following serum reactions were recorded during the course of the infection:

Aug. 21, or 15 days after injection:
B. abortus 80=1:10—1:100—0.
1:200—1:2000 + + +.
B. melitensis 2=1:10—1:60—0.
1:80—1:800 + + +.
B. melitensis 5=1:40—1:6000 + + +.
B. melitensis 3, 6 and 1=no agglutination.
Sept. 20, 1919, or 45 days after the injection:
B. abortus 80=1:2000 + + +, clumps.
B. abortus 14=1:40—1:80 neg. 1:100—1:1000 + + +.
Nov. 12, or 98 days after the infection:
B. abortus 80=1:80 + + +.
B. melitensis 655, 11 and 614=all negative.

In November, 1919, about 3 months after the intravenous injection of the abortus bacilli, the animal became suddenly ill and was found dead in his cage Nov. 12, 1919.

Postmortem Examination.—Body emaciated, weight 1,750 gm.; spleen small and firm; mesenteric lymph nodes slightly enlarged; tissues rather atrophic and dry; cause of death not determined.

Cultures from heart blood, liver, spleen, kidneys, lymph nodes and bone marrow contained numerous cocci and rods, but no B. abortus.

Exper. 3.—*Monkey* 12.—Pithecus syrichta (Linn.); female, weight, 1,960 gm. Repeated agglutination tests with serum were negative for B. abortus and B. melitensis. Inoculated Aug. 14, 1919, intravenously with ¼ slant or 12 billion B. abortus No. 80 isolated from liver of monkey 2. Temperatures on Aug. 15, 16, 17 and 18, normal. Monkey found dead at 4:00 p. m. Aug. 18, the fourth day after the injection.

Postmortem Examination.—Body well nourished; considerable fluid in abdominal cavity; spleen enlarged and rather soft; liver and kidneys congested; intestines omentum and mesenteric folds injected; lymph nodes in general slightly enlarged and soft. Heart muscle rather flabby, slight ventricular dilatation. Death was probably due to cardiac insufficiency.

Cultures were made from the heart blood, spleen, bone marrow, kidneys, bile, mesenteric, femoral and axillary lymph nodes. B. abortus was recovered from all the cultures except those made with heart blood and bile. The blood plates were densely crowded with colonies.

Exper. 1 must be considered in every respect successful; the monkey reacted to an intravenous injection of a moderately virulent strain of B. abortus by a marked production of specific agglutinins. The temperature curve, which represented the remittent type of pyrexia, resembled in many respects that reported on monkey 47 in Horrock's studies[7] on the conveyance of M. melitensis to healthy animals. The type of temperature was comparable to the one obtained in man, when the patient was suffering from what Shaw designated the "ambulatory" type of Mediterranean fever. The persistence of the infection, which in part explained the high agglutinin titer on the 51st day was proved by the extensive growth of B. abortus on the cultures prepared from the various organs. The observations on monkey 11, from a serologic standpoint, were similar to those noted in monkey 1. The infection exhibited itself primarily by an active production of agglutinins, although the temperature remained normal and the tissues on the 98th day after the injection were found to be free from B. abortus. The negative cultural finding of the tissues after death might in part be the result of the partial overgrowth of the plates and tubes by a coccus, which was probably responsible for the intercurrent disease. In view of the progressively diminishing serum agglutination reached before death, it appeared, however, possible that the monkey had recovered from the infection. Exper. 3 should be repeated in order to prove or disprove our conception that monkey 12 succumbed to a nonspecific injury to the heart muscle provoked by an intravenous inoculation of bacterial protein.

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[7] Report of the Commission on Mediterranean Fever, London, 1905, Part I, p. 50.

The experiments definitely indicate that monkeys are susceptible to B. abortus after intravenous inoculations and that these animals may show febrile reactions similar to those observed in monkeys infected with B. melitensis. The injection calls forth a noteworthy production of serum agglutinins and the bacteria persist for at least 50 days in the liver, spleen, kidneys and lymph nodes, while the blood stream sterilizes itself rapidly.

Infections Produced by the Feeding of Carrots, Bread, or Apples Contaminated with B. abortus of Low Virulence.—In order to prove some of the questions raised in the introduction, it was necessary to make certain experiments by introducing B. abortus into the body via the alimentary tract. Three problems suggested themselves in this connection, namely: 1. Was the single ingestion of a limited number of bacteria sufficient to cause an infection? 2. If not, what was the approximate number of infective feedings and quantity of bacteria necessary to produce a disease similar to malta fever? 3. What were the evidences of a successful feeding infection? It should be borne in mind in studying this problem with B. abortus, that, according to Eyre, McNaught, Kennedy and Zammit,[7] a B. melitensis infection, produced in the monkey by feeding, could not always be ascertained by the course of the animal's temperature or by its general appearance and behavior. In many instances, the postmortem findings and the demonstration of innumerable malta fever organisms in the tissues rudely contradicted the negative clinical or even the serologic observations.

Six monkeys were fed with B. abortus cultures; 5 received the growth of a strain of low virulence, while 1 ingested a highly pathogenic culture of porcine origin. The method of administration of the infective material was quite simple; the bacterial masses of well-grown cultures were smeared on sliced carrots, bread or apples and immediately offered to the animals, by whom the contaminated food was eagerly eaten. The following protocols detail the various experiments:

Exper. 4.—*Monkey* 1.—Pithecus syrichta (Linn.) male; weight, 2,625 gm. March 18 to April 2, 1919, well and active, serum repeatedly examined for B. abortus and melitensis agglutinins with negative results. April 3, 3:30 p. m., fed the growth of 2 peptic digest agar slants of a 48-hour old culture of B. abortus 752 (about 25 billion organisms) smeared on a sliced carrot. The monkey never suffered from fever and the serum reactions conducted on April 20, 28; May 7, 22; June 10 and 25, and July 8, failed to reveal specific agglutinins. On July 20 the animal developed severe diarrhea and was chloroformed in extremis on July 23, or 110 days after feeding the culture.

Postmortem Examination.—Body emaciated, weight 2,025 gm., catarrhal enteritis, enlarged mesenteric lymph nodes, slight spleen tumor and congestion of liver. Cultures were negative for B. abortus, but there was an extensive growth of various intestinal organisms from liver, spleen, lymph nodes and bone marrow. The heart blood was sterile.

The blood serum collected at necropsy agglutinated B. abortus 320 in a dilution of 1:10.

This experiment indicated that a single feeding of a fairly large dose of virus failed to produce an infection in the monkey. It was considered unnecessary to repeat this experiment as similar results were obtained in animals fed with artificially infected milk (exper. 11 and 12).

Exper. 5.— *Monkey* 3.—Pithecus syrichta, male, weight, 2,280 gm. Had been used in exper. 13, in which he had received repeated feedings of goat's milk containing large numbers of B. abortus, with negative results; blood serum repeatedly negative for B. abortus and B. melintensis. From Oct. 4 to Nov. 10, 1919, the monkey was fed daily the growth of one slant each of B. abortus 80 and 14, or an approximate total of 2,270 billion bacteria smeared on sliced carrots or bread. The serum tested Oct. 25, the twentieth day of feeding, was negative. The temperature was normal until Nov. 10, when it rose to 41.5 C. and remained at this level 24 hours. On the morning of Nov. 12, the 36th day of the experiment, the monkey was found dead.

Postmortem Examination.—The body was well nourished; mucous membranes were slightly anemic. Lungs showed numerous bronchopneumonic areas. The spleen was normal in size and consistency. Liver, kidneys, mesenteric, femoral and subscapular lymph nodes were normal.

Cultures of the lungs gave a pure growth of B. bronchisepticus; the lymph nodes and liver showed streptococci; while the blood plates smeared with the spleen pulp were covered with numerous typical B. abortus colonies together with a few streptococcus colonies. The heart blood was sterile, but the serum agglutinated B. abortus 80 in a dilution of 1:600 + + + and B. abortus 14 in a dilution of 1:800.

Exper. 6.—*Monkey* 13.—Ceropithecus philippinensis, female, weight, 2,225 gm. Aug. 5, 1919, well and active; serum negative for B. abortus and B. melitensis. Fed on August 5, 6, 7, 8, 9, 10, 11, 13, 14, 21, 24 the growth of one slant each of B. abortus 80 and 14 on carrots, bread or apples. An approximate total of 1,300 billion organisms were fed. Marked loss in weight. Temperature was never above 40 C.; the blood serum gave the following reactions:

First day of feeding—B. abortus 80=0.
B. melitensis 2=0.
Weight 2,225 gm.
Sixteenth day of feeding—B. abortus 80=1:40 + + +, 1:80 + +.
B. melitensis 2—0.
Twenty-second day of feeding—B. abortus 80=1:80 + + +.
B. melintensis 11=1:100 + + +.
Weight 1,965 gm.
B. abortus 14=1:100 + + +.
B. melitensis 655=1:100 + + +.
Weight 1,890 gm.

Aug. 26, had severe diarrhea and refused to eat. Aug. 27, no improvement; chloroformed.

Postmortem examination.—The cadaver was emaciated; 5 c c of fluid were found in the abdominal cavity; the mesenteric and the femoral lymph nodes were enlarged and rather soft. The spleen was enlarged, the pulp soft with enlarged follicles. The kidneys, the liver and lungs appeared normal. Numerous oesophagostomum nodules were present in the colon and cecum.

Cultures made from the liver, kidneys, urine, lung, bone marrow, femoral, iliac and mediastinal lymph nodes were sterile or contained a few cocci colonies. The plates smeared with the mesenteric lymph nodes were densely covered by typical colonies of B. abortus. The agar slants and the blood plates seeded with spleen pulp showed 11-20 colonies of B. abortus.

Exper. 7.—*Monkey* 33.—Pithecus syrichta (Linn.), male; weight, 2,575 gm. Agglutination tests with B. abortus 80; B. melitensis 7 and 11 were repeatedly negative. Feb. 11, 1920, white blood cells 16,400; hemoglobin, 68% Fed beginning March 9, 1920 (on 64 days, over a period of 115 days) one peptic digest agar slant each of B. abortus 80 (certified milk strain) and 14 (fetal membrane strain, 1907) on sliced carrots. Total organisms fed were estimated at 10,000 to 12,000 billion (75-80 billion per slant).

The temperature remained normal during the entire period of observation; there was a drop in the hemoglobin from 68% to 64%. The blood serum gave the following agglutination reactions:

March 9, or the 1st day of feeding—B. abortus 80 = 0; B. melitensis 11 =0; 7 = 0.
April 6, or the 28th day of feeding—B. abortus 80 = 0; B. melitensis 11 = 0.
April 20, or the 42nd day of feeding—B. abortus 80 = 1 : 100 + +; B. melitensis 11 = 1 : 60; 7 = 0.
May 3, or 55th day of feeding—B. abortus 80 = 1 : 100; heated serum also 1 : 100.
May 17, or 69th day of feeding—B. abortus 80 = 1 : 80.
June 1, or 84th day of feeding—B. abortus 80 = 1 : 100—200.
June 17, or 100th day of feeding—B. abortus 80=1 :80
June 30, or 113th day of feeding—B. abortus 80 = 1 : 100 + + +; 1 : 400 ±.
July 10, or 123 rd day of feeding—B. abortus 80=1 :1,000 +.
B. abortus, hog=1 :800 + +; B. melitensis 11 = 1 : 200 + + +.
B. melitensis 20 = 1 : 200 + + +; B. melitensis 9 = 0;
B. melitensis 615 = 0; B. melitensis 27 = 0.

July 10, the 123rd day after the first feeding and 8 days after the last feeding, the animal was chloroformed and the tissues cultured.

Postmortem Examination.—The cadaver weighed 2,510 gm.; the spleen was of normal size, rather hard and slightly nodular. The follicles were distinctly visible in the fairly dry pulp. The mesenteric, iliac, femoral, prescapular and mediastinal lymph nodes were not visibly enlarged; the liver, kidneys, and other organs appeared normal. The omentum was adherent to the appendix and portions of the colon; one of these adhesions enclosed an abscess, the size of a pea, containing brownish liquid pus. In the colon and appendix wall, numerous nodules the size of split peas, containing Oesophagostomum brumpti, were present. Cultures were made from the heart blood, spleen, bone marrow, liver, suprarenals, kidneys, lungs, inguinal, prescapular, mediastinal, mesenteric and iliac lymph nodes and from one abscess in the omentum and one oesophagostomum nodule. The blood plates smeared with the pus of these abscesses and the spleen pulp gave an abundant pure growth of B. abortus. All of the agar slants prepared with crushed liver remained sterile, with the exception of one tube, which showed 2 colonies of B. abortus. The other tissues proved to be sterile.

Exper. 8—*Monkey* 34.—Pithecus syrichta; female, weight 2,400 gm. Feb. 9 to April 6, 1920, repeated agglutination tests with unheated blood serum were negative for B. abortus. On April 6, the hemoglobin was 80% (Sahli) and there were 14,700 white blood cells. The animal was fed on 82 days, over a period of 142 days, one slant each of B. abortus 80 and 14, or a total of 14,000 to 16,000 billion bacteria smeared on bread or sliced carrots. Feeding was stopped on July 29, 1920. Weight, blood counts, temperature and agglutination reactions were determined at varying intervals. The temperature never was above 40 C., and the hemoglobin factor showed normal fluctuations. The blood serum always gave negative serum reactions with B. abortus and B. melitensis antigens. The animal is still alive and in excellent health.

In order to determine the possibility of an elimination of B. abortus in the feces of monkeys heavliy fed with abortion bacilli, guinea-pigs were inoculated subcutaneously with emulsions of stools, which had been collected and prepared on the 36th, 45th and 52nd days of the experiment. The animals were sacrificed on the 50th to 60th day after the inoculations. Characteristic lesions were absent and no abortion bacilli were recovered from their spleens.

Monkeys 3, 13 and 33 are examples of successful feeding infections with repeated and enormous doses of B. abortus administered on carrots, bread or apples. The cultures used in these 3 experiments were of bovine origin and of moderate virulence as judged by the lesions they produced in guinea-pigs. Monkey 3 had been fed previously for a period of 19 days with naturally infected milk, but failed to show signs of infection. The positive cultural findings at necropsy and the specific and high agglutination reactions can be safely attributed to the ingestion of the cultures. Unfortunately, the observations on monkey 3 were somewhat invalidated by the death of the animal from bronchopneumonia. During the summer vacation monkey 13 was attended by an inexperienced caretaker, and lack of proper cleanliness probably resulted in the development of the severe diarrhea. It is, however, evident from the presence of specific serum agglutinins on the 16th day after the beginning of the administration of abortus cultures, that the monkey was infected previous to the occurrence of the enteritis. The latter can, therefore, not be considered a contributory factor to the abortus infection.

Monkey 34 illustrates the fact that the ingestion of enormous doses of B. abortus is not always followed by an infection, provided the appearance of specific agglutinins is to be chosen as the only reliable antemortem criterion. The necropsy findings of its mate, monkey 33, therefore deserve further discussion. The large intestines and the cecum of this animal were seriously injured by numerous abscesses due to oesophagostomum and the pathologic processes extended into the mesentery and omentum. The majority of these parasitic nodules harbored enormous numbers of viable abortion bacilli. In reviewing the necropsy reports of the monkeys successfully infected by the ingestion of B. abortus, it was noted that monkey 13 also showed oesophagostomum nodules, while monkey 3 was not infected. *Special attention has been given to the significance of this observation in subsequent experiments, but true infections with the B. abortus have been recorded in animals which showed no coincidental intestinal lesions due to parasites.* It is therefore only remotely possible that the parasitic lesions predispose to an invasion of B. abortus via the alimentary canal in monkeys. In future experiments animals which are free from round worms should be chosen. In our experience with 80 monkeys bought during the last 3 years, such a prerequisite is probably not readily obtainable with the animals at present available on the market. These conditions are mentioned because the British Commission seriously considered the possibility that an absolute continuity of the mucous membrane of the alimentary tract of monkeys may in some cases prevent the development of a feeding infection with B. melitensis.

The presumptive evidence of infection has never manifested itself by the presence of clinical symptoms, and the course of the animals' temperatures has been constantly normal. The duration of the incubation period and the course of the infection could therefore only be judged by the appearance and degree of the specific agglutinins, which developed in the blood serum of the monkeys. In this connection it should be emphasized that the serum of monkey 3 on the 20th day and of monkey 33 on the 28th day of feeding failed to agglutinate B. abortus, while on the 36th and 42nd day, respectively, the serum of these 2 monkeys was distinctly positive. These observations correspond in many respects to those recorded by Horrocks, Kennedy and Shaw [8] on monkeys infected by the ingestion of B. melitensis; approximately 29 days elapsed after the feeding of the cultures before agglutinins were demonstrable in the blood serum. This delayed appearance of the agglutinins lends additional support to the contention that the parasitic processes of the intestines do not favor the tissue invasion of the ingested B. abortus. For example, in monkey 3, which had no nodules of oesophagostomum in the colon, agglutinins were absent on the 20th day, while similarly in the parasitized monkey 33, 29-30 days elapsed before positive serum tests were obtainable. They were absent at least 28 days after the beginning of the experiment.

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[8] See table in Report of Commission, 1907, Part 6, p. 46.

In those monkeys in whom serologic evidence of infection was present following the introduction of B. abortus by the alimentary route, absolute proof of pathogenicity was obtainable in every instance by the isolation of the organism, at least from the spleen, if not from numerous other viscera.

In order to test the assumption that a strain of B. abortus, which is usually fatal in relatively small amounts to guinea-pigs, possesses also greater invasive properties for monkeys than a less virulent strain, two additional experiments were carried out. The B. abortus strain employed had been isolated by Dr. J. Traum from the liver of an aborted hog fetus and had proved very lethal for guinea-pigs;. it grew profusely on glycerol peptic digest agar and proved biochemically and serologically a typical B. abortus.[9]

Exper. 9—Monkey 43.—Pithecus syrichta (Linn.); female, weight 2,375 gm.; healthy, with a blood serum negative to B. abortus and B. melitensis, was fed on 43 alternate days from June 1, to July 29, 1920 (over 58 days) the growth of one slant of B. abortus (hog strain, isolated by Traum) cultivated from the liver of monkey 40. The individual dose averaged 60 billion and for the entire feeding period a total of 2,580 billion bacteria were fed on sliced carrots. The temperature remained normal, but there was a decided loss in weight and specific agglutinins developed in the blood serum as follows:

1st day—weight 2,775 gm.—agglutination with B. abortus=0.
16th day—weight 2,450 gm.—agglutination with B. abortus=1:80 + + +.
37th day—weight 2,425 gm.—agglutination with B. abortus=1:400 + + +.
55th day—weight 2,100 gm.—agglutination with B. abortus=1:800 + + + to 1:1,000 + +.
58th day—feeding stopped.
65th day—weight 2,225 gm.—agglutination with B. abortus=1:600 + + + to 1:2,000 +.

The monkey exhibited no clinical symptoms of infection and was chloroformed on Aug. 4, 1920.

Postmortem Examination.—Body well nourished; spleen slightly enlarged and nodular, pulp rather dry; liver normal in size and color; inguinal, mesenteric, subscapular and mediastinal lymph nodes slightly enlarged and slightly pigmented; both kidneys and bone marrow normal. Principle lobe of the left lung contained an echinococcus cyst the size of a plum. In the colon were numerous oesophagostomum nodules with adhesions to the omentum. Cultures were made from the spleen, liver, kidneys, bile, bone marrow, femoral, mesenteric, mediastinal and subscapular lymph nodes, several oesophagostomum abscesses, urine and heart blood. One of 3 plates spread with spleen pulp gave 2 colonies and one plate of 2 smeared with the juice of the mesenteric lymph nodes showed 8 colonies of B. abortus. All the other cultures were sterile or contained a few staphylococci; on the lung plates B. bronchisepticus colonies were present.

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[9] Jour. Infect. Dis., 1920, 27, p. 185.

Exper. 10—Monkey 44.—Pithecus syrichta (Linn.); male, weight 2,800 gm.; healthy with a blood serum negative to B. abortus and B. melitensis; was fed on 43 alternate days from June 1, 1920, to July 29, 1920 (58 days), the growth of one slant of B. abortus (hog strain) isolated from monkey 40. The method of feeding was the same as used for monkey 43. The temperature remained normal; there was a slight loss in weight and the agglutinins developed in the blood serum as follows:

1st day—weight 2,800 gm.—agglutination with B. abortus = 0.
16th day—weight 2,600 gm.—agglutination with B. abortus = 1:40 +.
37th day—weight 2,700 gm.—agglutination with B. abortus = 1:100 + + +;
55th day—weight 2,750 gm.—agglutination with B. abortus = 1:800 — 1:1,000 + + +.
58th day—feeding stopped.
65th day—weight 2,925 gm.—agglutination with B. abortus = 1:800 + + +.
81st day—weight 3,000 gm.—agglutination with B. abortus = 1:800 + + +.
B. melitensis 27=1:600 +++.
213th day—weight 3,125 gm.—agglutination with B. abortus = 1:80 + + +.
325th day—weight 3,100 gm.—agglutination with B. abortus = 1:10 + + +.

On May 5, 1921, the animal was still alive and in excellent condition.

Monkeys 43 and 44 present some interesting features. Judging by the appearance of the serum agglutinins, both monkeys became infected sooner than those which had been fed with less virulent abortion bacilli. The degree of infection did not manifest itself in the necropsy cultures of monkey 43; very few bacteria grew on the plates prepared from the spleen and mesenteric lymph nodes. In connection with the previous discussion particular attention must be concentrated on the fact that the pus material of several oe ophagostomum nodules did not contain B. abortus; they were either sterile or contained a few staphylococci. Monkey 44 gradually recovered from his infection and regained his original weight. The blood serum agglutinins returned to a low level, but even after a lapse of 325 days, a strongly positive reaction in a dilution of 1:10 was readily obtained. These two experiments indicate that a fairly high percentage of monkeys can be successfully infected via the alimentary tract when large and repeated doses of B. abortus are fed on sliced carrots, bread or apples. It has been demonstrated that these infections are associated with strongly positive agglutination reactions and that B. abortus can regularly be isolated from the spleen and other organs. The serum of the infected monkeys agglutinates B. melitensis cultures in slightly lower dilutions than B. abortus. In order to prove definitely the nature of the infection, it is necessary to conduct absorption tests as stated in article 2 of this series.

Feeding Experiments with Cow's Milk Artificially Infected with B. abortus.—In a further attempt to investigate the factors involved in the infection through the alimentary canal, 4 monkeys were fed on pasteurized milk, which had been artificially contaminated with varying amounts of 2 bovine B. abortus cultures, Nos. 80 and 14. The strains used in these experiments were the same as those used in the successful experiments 6 and 7.

Exper. 11—Monkey 35.—Pithecus syrichta (Linn.); female, weight, 2,500 gm. Feb. 9 to March 9, 1920, repeated agglutination tests with unheated serums were negative for B. abortus and B. melitensis 7 and 11; the hemoglobin was 70% and the white blood cells 17,400. The animal received in a pannikin on 52 days over a period of 80 days, 100 c c of pasteurized cow's milk to which had been added on each occasion, just before feeding, the growth of one slant of B. abortus 80 and 14. A total of about 10,000 billion bacteria had been given in the milk. As a rule, the monkey received this mixture late in the afternoon, after a starvation period of 18 to 20 hours and usually lapped up from ½ to ⅔ of the amount offered. Inside of from 30 to 60 minutes, the pannikin was ordinarily found to be empty; the animal received then its regular meal.

The monkey never suffered from fever, and gained in weight during the experiment. Agglutination tests made at regular intervals were always negative. The animal is still alive and in good health.

Monkey 36.—Pithecus syrichta (Linn.); female, weight, 2,450 gm. This animal was treated for the same period in a similar manner as monkey 35, with negative results. The serum tests with B. abortus antigens were regularly negative.

Exper. 12—Monkeys 37 and 38.—Monkey 37: Pithecus syrichta (Linn.); female, weight, 2,075 gm., had been under observation since Feb. 9, 1919; serum tests failed to give positive reactions with B. abortus and B. melitensis antigens. The monkey was fed by a similar procedure as monkeys 35 and 36 for 44 consecutive days with 100 c c of pasteurized cow's milk, to which had been added the growth of 1/100 slant of B. abortus 80 and 14. A total of about 80 to 100 billion bacteria were consumed by the animal during the experiment. She never suffered from fever and never gave specific serum reactions.

On May 14, 1920, or on the 65th day of the experiment, or 21 days after the last feeding of artificially infected milk, the monkey received 0.3 gm. of cascara and the growth of one slant each of B. abortus 80 and 14 on carrots for 10 consecutive days; then magnesium sulphate in milk for one day and aloin pills (2, then 4, and then 6 pills of ¼ grain daily) for 7 days, together with carrots artificially infected with B. abortus cultures. Neither catharsis nor the appearance of specific agglutinins could be provoked.

From July 8 until Aug. 19 (from the 121st to the 163rd day) the monkey received daily 25 c c of goat's milk collected from goat 2. This milk contained very few abortion bacilli, but possessed a high agglutinin content. During this period of the experiment and for several months subsequently, the monkey never had fever, and the blood serum gave negative agglutinin reactions with B. abortus and B. melitensis antigens.

Monkey 38.—Pithecus syrichta (Linn.); a female, weighing 2,000 gm., had been under observation since Feb. 9, 1919; the serum had repeatedly been negative with B. abortus and B. melitensis antigens. This animal was fed infected milk in the same manner and on the same dates as monkey 37. Instead of cascara or aloin, tincture rhubarb was administered as a cathartic and 35-40 c c of goat's milk were given on 42 days over a period extending from May 13 to July 8, 1920.

The monkey never suffered from fever, gained slightly in weight and the blood serum developed no specific agglutinins for B. abortus.

It is evident from the data presented that monkeys 35, 36, 37 and 38 failed to become infected. Repeated observations gave the impression that the animals drank a considerable portion of the contaminated food. It was therefore surmised that monkeys 35 and 36 ingested practically the same number of bacteria as those which had been fed with contaminated carrots. The results, however, indicate that this was not the case; in all probability a larger portion of the milk was spilled than was actually recorded, and the total number of B. abortus consumed was less than calculated. In no other manner can one explain why organisms ingested in milk should be less pathogenic than those fed on vegetables. As the repeated feeding of milk containing two slants of bacteria was ineffective, it was not anticipated that 1/100th of a slant would be sufficient to cause an infection, so that the negative results in monkeys 37 and 38 were not surprising. There is a remote possibility that these monkeys were refractory. One recalls in this connection that the British Commission succeeded repeatedly in infecting monkeys with B. melitensis after the ingestion of a single quantity of mixed milk containing not more than 5,000 specific bacteria. Monkeys 35, 36, 37 and 38 received and probably ingested with impunity many thousand times the amount necessary to cause a typical undulant fever infection.

These experiments demonstrate another fact. The pasteurized milk contained specific agglutinins; its whey clumped B. abortus in a dilution of 1:200. The milk of goat 2 (see appendix), which was fed for 42 days to monkeys 37 and 38, was similarly potent. On one occasion, the secretion of the right infected udder agglutinated the B. abortus in a dilution 1:2,000. Irrespective of the ingestion of this milk, rich in antibodies, neither of the monkeys presented any specific agglutinins in the blood serum. The mere ingestion of milk containing antibodies does not lead, at least in the monkey, to an appearance of agglutinins in the blood serum; only an invasion of the tissues, limited and transitory as it may be, apparently can be responsible for the formation of specific serum agglutinins.

Feeding Experiments with Naturally Infected Goat's Milk.—In this series of experiments an attempt was made to reproduce the conditions as they were found to exist by the British Commission in their feeding experiments with milk obtained from goats naturally infected with B. melitensis. It was originally planned to pass various strains of B. abortus successively through the udders of several goats. In this manner it was hoped to transform the bovine into a caprine strain of B. abortus and perhaps to produce an organism pathogenically even more closely related to the B. melitensis than previous observations had demonstrated. On account of lack of available goats, this phase of the experiment has been temporarily abandoned. The few animals infected with B. abortus furnished milk specimens, which were in many respects similar to those found in malta fever goats. The most important observations dealing with the goats are presented in the appendix to this paper. It is emphasized that the milk repeatedly contained as many as 100,000 to 250,000 viable abortion bacilli per c c. In order to obtain these figures, the milk was plated in suitable dilutions in peptic digest liver agar. It is not unlikely that a large number of viable bacteria failed to grow on the poured plates, irrespective of the fact that the B. abortus strains employed grew ordinarily freely in the presence of oxygen. The figures obtained should therefore be considered as averages; it is readily possible that 1-2 million per c c would be nearer the true content of the milk than the 250,000 recorded. The mid milk and the strippings, after having discarded the first 5 c c, were either sterile or gave a pure culture of B. abortus. The milk obtained from goat 1, which was infected on 3 occasions with 2 different strains of B. abortus, and goat 2, injected once with 3 recently isolated bovine strains were therefore remarkably similar to that ordinarily collected from malta fever goats, and feeding experiments on monkeys, therefore, suggested themselves. The experiments thus far completed are briefly as follows:

Exper. 13—Monkey 3.—Pithecus syrichta; male, weighing 2,150 gm., July 2, 1919, was well and active; the serum was negative for B. abortus and B. melitensis. The animal received for a period of 39 days, in a pannikin, 80-200 c c of milk obtained from goat 1 (see appendix). A total of 2,090 c c of milk were placed in the cage, but the monkey spilled the greater part of the fluid and rarely drank more than 20-30 c c at a time. The milk contained from 50-100,000 B. abortus per c c, and the whey of the right udder agglutinated B. abortus in a dilution of 1 : 1,000. The monkey never had any rise in temperature, and the blood serum when tested on July 18 and Aug. 12 failed to agglutinate B. abortus.

Exper. 14—Monkey 19.—Macacus cynomolgus; male, weight 1,650 gm. Sept. 20 to 30, 1919, serum was repeatedly tested for B. abortus and B. melitensis agglutinins with negative results.

From Sept. 30 to Oct. 30, 1919, the monkey received on 26 week days 60-100 c c of goat's milk (goat 1), obtained from the right uninfected half of the udder. At the beginning of the experiment, the milk from the infected left half of the udder averaged 30-60 c c, was rich in leukocytes and the sediment of a centrifugalized specimen gave when cultivated on glycerol agar slants numerous colonies of B. abortus. Beginning Oct. 12, the secretion of the diseased udder was very scanty, about 5-10 c c and toward the end of the experiment (Oct. 14, 1919) the monkey was fed entirely with milk obtained from the right half of the udder, which agglutinated B. abortus in a dilution of 1:500. The temperature of the monkey remained normal throughout the experiment. Serum tests conducted on Oct. 10 and 25 were negative for B. abortus and B. melitensis. Nov. 8, 1919, after showing symptoms of respiratory disease for 3 days, the monkey was found dead.

Postmortem Examination.—Body emaciated, tissues dehydrated; lungs, numerous bronchopneumonic areas; spleen tumor; liver and kidneys mottled and viable. Mesenteric and femoral lymph nodes slightly enlarged and soft.

Cultures made on blood plates with the heart blood, spleen, liver, bone marrow, kidneys, urine and bile failed to reveal colonies of B. abortus. The lungs, bone marrow, spleen and kidneys revealed hemolytic streptococci.

The experiments clearly indicate that B. abortus infection is not readily accomplished in monkeys fed naturally infected goat's milk. Monkeys 3 and 19, which had been fed for 12-39 days, respectively, the milk of goat 1 failed to develop specific serum agglutinins. One, monkey 3, was definitely not refractory because when subsequently fed B. abortus on carrots he became infected and showed agglutinins in the serum and bacteria in the tissues. In the light of previous observations, the negative results on these two monkeys were probably due to the following factors: 1. The two abortus strains used for the infection of the udder of goat 1 were low in virulence. 2. The numbers of viable abortion bacilli in the milk in comparison to those employed in the carrot feeding experiments were very small. In all probability the daily elimination of bacteria in the milk fluctuated considerably as the figures obtained by direct plating clearly demonstrated. The milk fed to monkey 3 probably contained toward the end of the experiment very few abortion bacilli; thus it was noted that guinea-pigs fed with the same samples of milk failed to contract abortion disease; whereas earlier in the experiment, the milk contained a sufficient number of B. abortus to infect guinea-pigs easily by feeding. Moreover, both monkeys rarely consumed more than one-half of the milk offered; monkey 3 receiving at least at the beginning of the experiment milk specimens, which were teeming with abortion bacilli, spilled the greater portion of the fluid. The negative outcome of these two experiments supports the previous conclusions, that monkeys cannot be infected via the alimentary tract with a relatively small number of abortion bacilli of low virulence even when the contaminated food is administered for more than 30 days. B. abortus, therefore, differs fundamentally from B. melitensis.

Two additional monkeys, 37 and 38 (see data of experiment 12), were fed for 42 days with milk collected from goat 2. It is known that the secretion of this animal contained very few abortion bacilli; the negative outcome of these feeding experiments is probably due to the factors already discussed.

Being impressed by the high virulence for guinea-pigs of a strain of B. abortus recently isolated from a hog fetus, it suggested itself to reinfect goat 1—which was again in a period of lactation having apparently recovered from the original infection with B. abortus 80—with this hog strain and to conduct further feeding experiments on monkeys. These tests are briefly as follows:

Exper. 15—Monkey 40.—Pithecus syrichta (Linn.); female, weight 2,725 gm., had been under observation since Jan. 12, 1920. It had been immunized with living typhoid bacilli, and the blood serum agglutinated B. typhosus in a dilution of 1:8,000, but was negative for B. abortus and B. melitensis. The hemoglobin was 73% and the white blood cells 12,800.

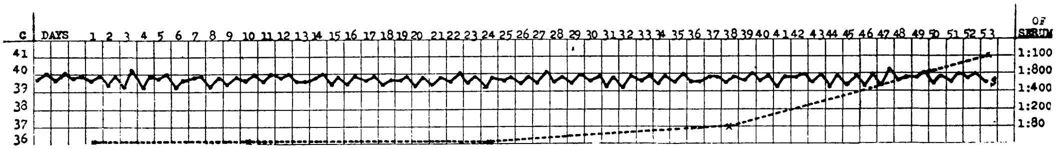

Chart 2 (Monkey 40).—Fed with goat's milk naturally infected with B. abortus of porcine origin.

From March 30 to May 22 the monkey received on 46 successive days 50-100 c c of goat's milk (goat 1), which contained from 20-250,000 abortion bacilli (strain hog) per 1 c c. A total quantity of 2,862 c c of infected milk were offered to the animal in a pannikin. Repeated observations indicated that less than 20 c c were spilled by the animal daily. The method and the time of feeding were those stated for monkey 35. It was estimated that the monkey consumed daily between 1,500 and 2,800 million highly virulent abortion bacilli. On April 23, on the 24th day of feeding, the milk from the right udder of the goat contained 1,500 abortion bacilli per c c and from the left udder 250,000 organisms per c c. May 10, or on the 40th day of the experiment, the milk from the right udder contained 30,000 organisms per c c and from the left udder 180,000 organisms per c c. (For further details see the history of goat 1 in appendix 1.)

The temperature of the monkey showed slight fluctuations during the entire period of observation; it never exceeded the normal average of 40.2 C. (Chart 2). There were no striking clinical symptoms but loss in weight. The figures for the hemoglobin percentages, and the agglutination reactions were as follows:

1st day—weight 2,725—hemoglobin 73%—agglutination with B. abortus = 0.
10th day—weight 2,700—hemoglobin 73%—agglutination with B. abortus = 0.
24th day—weight 2,650—hemoglobin 60%—agglutination with B. abortus = 0.
38th day—weight 2,550—hemoglobin 65%—agglutination with B. abortus =
1:80 + + +.
50th day—weight 2,350—agglutination with B. abortus=1:800 + + +.
1:800 + + +.
53rd day—weight 2,300—agglutination with B. abortus=1:800—1:1,000 + +.
B. melitensis 11 =
1:400 + + +

May 1, emulsion of stool was injected into 2 guinea-pigs; when sacrificed 3 months later, these animals were free from abortion disease. On the 53rd day, or 2 days after the last feeding of infected milk, the monkey was chloroformed and cultures carefully made.

Postmortem Examination.—Cadaver in fair condition; the omentum showed old adhesions to the colon. The spleen weighed 7.2 gm., was distinctly enlarged, and firm and exhibited numerous large follicles. Mesenteric, inguinal, subscapular, mediastinal and iliac lymph nodes were enlarged and hard. Liver and kidneys normal; no intestinal parasites or oesophagostomum nodules. In the lung a few small hemorrhages. Cultures were made from the spleen, inguinal, mediastinal, mesenteric, subscapular and iliac lymph nodes, liver, bile, kidneys, urine, bone marrow, lungs and heart blood. The blood plates smeared with the sections or juices of all the organs were densely crowded with B. abortus colonies. The heart blood was enriched in broth and gave on plating a profuse growth of abortion bacilli. The isolated bacteria were promptly agglutinated by a specific abortus serum.

Microscopic examination of the spleen revealed dilated sinuses with a distinct hypertrophy of the sinusoidal cells; the increase of the splenocytes and lymphocytes in the follicles was marked. The malpighian bodies exhibited degenerative changes. In the lymph nodes, desquamated pulp cells and loose endothelial cells in the cortex, together with a pronounced increase of the lymphocytes, attracted attention. Section of the liver, bone marrow and kidneys failed to reveal pathologic changes.

Exper. 16—Monkey 42.—Pithecus syrichta (Linn.); female, weight 2,425 gm. On June 1, 1920, the animal was in excellent health; the blood serum gave no reaction with B. abortus and B. melitensis. For 52 days, over a period of 85 days, the monkey was fed daily 100 c c, or a total of 5,200 c c. of infected milk of goat 1. At the beginning, the animal refused to drink the milk without the addition of sugar; at least one-half of the contents of the pannikin was spilled. In the second week of the experiment, the monkey consumed practically two thirds of the goat's milk placed in the cage.

June 1, or the 1st day of the feeding, the milk from the left udder of the goat contained 11,800 abortion bacilli per c c and from the right udder 2,500 organisms per c c; the milk whey of the left udder agglutinated B. abortus in a dilution of 1:200 and from the right udder in a dilution of 1:600. On June 25, or on the 25th day of the experiment, the milk of the left udder was sterile, and the milk of the right udder contained 20 abortion bacilli per c c. July 24, on the 53rd day of the experiment, the milk of the right udder was sterile, while the milk of the left udder contained 10 organisms in the sediment of the centrifugalized 25 c c milk sample; the mixed milk of both udders agglutinated B. abortus in a dilution of 1:1,000. On August 19, or on the 79th day of the experiment, the milk of the left udder was sterile; the sediment of 25 c c of milk of the right udder gave a few colonies; the whey agglutination was positive in a dilution of 1 : 200.

The serum of the monkey was tested for agglutinins on June 16, July 7 and 28 and August 14, with negative results. There was steady gain in weight and normal temperature throughout the period of observation. The animal died Dec. 20, 1920, while being bled from the saphenous vein.

Postmortem Examination.—Status thymo-lymphaticus; old adhesive left pleurisy; no intestinal parasites. Cultures from spleen, mesenteric, inguinal and subscapular lymph nodes, liver, bone marrow, sterile. Serum gave a partial agglutination to B. abortus in a dilution of 1 :60 in unheated serum; negative in heated serum.

The observations made on monkey 40 present several important facts. The ingestion of virulent abortion bacilli supplied in the naturally infected milk of a goat may lead to a generalized invasion of the tissues and even the heart blood. The intestinal tract of monkey 40 was entirely free from parasites or parasitic lesions. The bacterium penetrated the intact mucous membrane. The duration of the incubation period was more than 30 days. The infection manifested itself in the appearance of specific blood serum agglutinins, a loss in weight and a secondary anemia (Basset-Smith[10] reports the constant occurrence of a secondary anemia in undulant fever) with an increase in lymphocytes, while the animal's temperature remained normal, irrespective of the fact that the tissues were teeming with B. abortus. Monkey 42, on the other hand, which also received the naturally infected milk of goat 1, at a later date, showed no signs of an infection throughout the course of the experiment; no traces of agglutinins were demonstrable in its blood serum even up to the date of the termination of the feeding on the 85th day. On the 202nd day, when the monkey died on account of a status thymo-lymphaticus, the unheated serum agglutinated B. abortus in a dilution of 1 :60, but specific bacteria could not be isolated from the tissues. The failure to contract an infection was probably due to the fact that the monkey refused to take a sufficient quantity of the infected milk when it was particularly heavily contaminated with abortion bacilli. When the monkey had been trained to drink from 20-40 c c of milk, the total daily number of abortion bacilli consumed was obviously insufficient to cause an infection via the alimentary canal. Exper. 16, therefore, suggests that the ingestion of goat's milk has no predisposing influence in the intestinal invasion of abortion bacilli; it, however, proves by contrast with exper. 15 the previously stated conclusion that only enormous doses of abortion bacilli repeatedly ingested are capable of producing infections in monkeys.

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[10] Jour. Hyg., 1912, 12, p. 497.

Feeding of Pasteurized Cow's Milk Artificially Reinforced with Autolyzed Abortion Bacilli.—The observations of Larson, Sedgwick and Ramsey,[11] of Nicholl and Pratt[12] and of Cooledge[13] on the presence of specific (?) agglutinins for B. abortus in the serum of man, suggested further feeding experiments on monkeys. Cooledge stated that he caused the appearance of B. abortus antibodies in the blood serum of adults by feeding milk, which was naturally infected with B. abortus and which contained B. abortus antibodies. His explanation that these agglutinins and fixing antibodies resulted from an absorption in the large intestines of those present in an infected cow's milk appeared to be not entirely justified. These conclusions drawn by analogy from the experiments of Ehrlich and Wassermann,[14] dealing with the assimilation of antitoxin through the intestinal canal, should not, as will be shown later, be applied without qualification to this problem. In searching for a better interpretation of these serum reactions in man, it suggested itself to investigate the absorption of bacterial proteins through the intestinal tract of monkeys. Cow's, as well as goat's milk, obtained from infected animals, may contain considerable amounts of autolyzed abortion bacilli, which may even exceed the number of viable invasive organisms. In order to prove that in the monkey at least the development of specific agglutinins is not caused by the assimilation of these substances, the following experiments were carried out:

Exper. 17—Monkeys 35 and 36.—These monkeys, used in Exper. 11, with negative results, were fed for 20 days, over a period of 40 days, with pasteurized cow's milk, artificially contaminated with heat killed B. abortus, strain "hog." The cultures were prepared on glycerol peptic digest agar, washed off with distilled water and heated for 2 hours at 60 C. Approximately 65 billion dead bacteria were added to the milk of each monkey. Both animals had been trained to drink the milk and spilled less than 20 c c of the total amount of 50 c c placed in the cage. Serum tests were made every 10 days for 60 days, but no specific agglutinins were recorded.

It is obvious from these experiments that the ingestion of heat killed, partially autolyzed abortion bacilli is not responsible for the appearance of specific agglutinins in the blood serum of monkeys.

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[11] Amer. Jour. Dis. Child., 1915, 10, p. 197.

[12] Ibid., p. 2033.

[13] Jour. Med. Res., 1916, 29, p. 459; Mich. Agric. Exper. Station, 1918, No. 33.

[14] Ztschr. f. Hyg. u. Infektionskrankh., 1892, 12, p. 183.

Such immune bodies are apparently only created as a result of a definite tissue invasion by virulent B. abortus and their presence is, therefore, indicative of a true infection.

EXPERIMENTS WITH B. MELITENSIS

In the three preceding papers of this series, it has been pointed out that the distinction between B. abortus and B. melitensis not infrequently offers great difficulties. Particularly in connection with the B. melitensis infection experiments on guinea-pigs, it has frequently been impossible to state without conducting extensive absorption tests whether a strain of gram-negative bacilli which is agglutinated by a potent B. melitensis or B. abortus serum, is really a B. melitensis.

In order to prove the melitensis character of two of these strains, which were pathogenic for guinea-pigs on intratesticular injection, the following experiments were carried out:

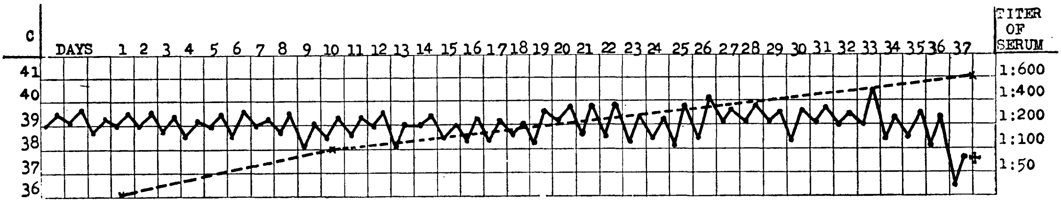

Chart 3 (Monkey 14).—Subcutaneous inoculation of B. melitensis.

Exper. 18—Monkey 14.—Macacus cynomolgus; male, weight 2,830 gm. Aug. 19, 1919, well and active; serum negative for B. abortus and B. melitensis. Aug. 21, inoculated subcutaneously on abdomen with one-half slant of B. melitensis 655 (originally isolated from the precrural lymph node of guinea-pig 655, which had been infected with a culture obtained from the urine and testes of guinea-pig 614, previously injected with B. melitensis, strains 1, 2, 3 and 4). Temperature reaction was distinctly remittant in character (See chart 3). On Sept. 1, or on the 11th day after the inoculation, the blood serum gave the following reactions:

B. melitensis 655 = 1 : 100 + + +
B. melitensis 7 = 0
B. abortus 80 = 1 : 40 + + + ; 1 : 100 +

The monkey appeared lethargic and toxic on Sept. 15; he lost in flesh and was dying Sept. 27; killed with chloroform.

Postmortem Examination.—Body much emaciated (weight 2,250 gm.), spleen small, hard and fibrous; pulp dark brownish; liver slightly mottled; lymph nodes not appreciably enlarged; femoral lymph nodes rather firm; intestinal content frothy; lungs normal.

Cultures were made from the spleen, heart blood, liver, bile, kidneys, bone marrow, mesenteric, femoral, axillary and mediastinal lymph nodes. B. melitensis was recovered from the spleen, right kidney and liver. The blood plates smeared with spleen pulp showed about 100 colonies, those of the right kidney had 20, and those made from the liver gave 2.

The blood serum collected at necropsy, or 37 days after the injection, gave the following reactions:

B. melitensis 655 = 1 : 600 + + +.
B. abortus 60 = 1 : 600 + + +, after absorption with B. abortus 80, serum agglutinated B. melitensis 1 : 400 + + +; the reverse test resulted in negative agglutination with both strains.
B. melitensis 8, 11 and 2—were not agglutinated in dilution 1 : 40 — 1 : 1,000.

Exper. 19—Monkey 15.—Pithecus syrichta (Linn.); male, weight 2,010 gm. Aug. 20 to 28, 1919, well and active, temperature normal; serum repeatedly examined for B. abortus and B. melitensis agglutinins with negative results. Aug. 28, inoculated intravenously with one-quarter slant of B. melitensis 655 (precrural lymph node of guinea-pig 655) by way of right saphenous vein. Temperature was intermittent in character (see chart 4). On Sept. 1, the monkey was noticeably toxic, and had diarrhea. On Sept. 20 a similar reaction was noticed. The blood serum gave the following reactions:

Sept. 10, 1919, or 13th day after infection:

B. abortus 80=1 :2,000 + + +.
B. abortus 14 = 1 : 600 + + +
B. melitensis 655 = 1 : 400 + + +; 1 : 2,000 +
B. melitensis 7 = 0

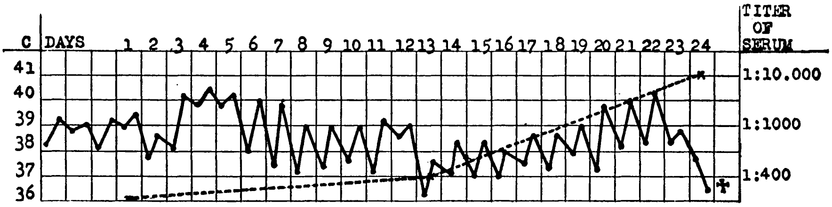

Chart 4 (Monkey 15).—Intravenous inoculation of B. melitensis.

Sept. 20, 1919, or 23rd day after infection:

B. abortus 80 = 1 : 20.000 + + +
B. abortus 11 = 1 : 10.000 + + +
B. melitensis 655 = 1 : 10.000 + + +
B. melitensis 2 = 1 : 1,000
B. melitensis 7 and 9 = 0

On Sept. 21 the animal was comatose and dying. It was chloroformed.

Postmortem Examination.—Body emaciated (weight 1,380 gm.); anus soiled with liquid fecal matter; spleen small, reddish, fibrous pulp; liver light brown and mottled. Mesenteric, femoral and axillary lymph nodes slightly enlarged and soft; bone marrow deep red, like raspberry jam, soft. Blood coagulated rapidly; no intestinal parasites; kidneys light colored. Cultures were made from the spleen, heart blood, liver, kidneys, bone marrow, lung, bile, urine, femoral, axillary and mesenteric lymph nodes. The blood plates smeared with the spleen, bone marrow of left and right femur and one liver lobe were densely crowded with typical colonies; the left lung and urine showed only a few colonies. Absorption tests proved the bacteria to be B. melitensis, group 2.

A study of the microscopic preparations revealed a hyperplasia of the sinusoidal cells similar to that usually seen in an early typhoid infection. The spleen showed slight fibrosis and pigmentation, the latter probably the result of an extensive destruction of the blood cells.

Exper. 20—Monkey 46.—Pithecus syrichta (Linn.); female, weight 2,400 gm. July 27, 1920, injected subcutaneously on left shoulder, 9 billion B. melitensis 27 (strain passed through and isolated from guinea-pigs 880 and 881). The temperature remained normal throughout the period of observation of 94 days. The serum reactions were as follows:

1st day—agglutination with B. abortus 80 = 0
B. melitensis 27 = 0
24th day—agglutination with B. melitensis 27 = 1:200 + + +
B. abortus 80 = 1:400 + + +
94th day—agglutination with B. melitensis 27 = 1:800 + + +
B. abortus 80 = 1:800 + + +

The monkey was chloroformed on Oct. 25, or 94 days after the injection of the culture.

Postmortem Examination.—Body well nourished; spleen small and rather light in color; other organs normal. The omentum, adherent to the cecum, contained two small abscesses; numerous esophagostomum nodules in colon and cecum.

Cultures from kidneys, spleen, bone marrow, urine, liver and lymph nodes sterile; plates smeared with pus from the omental abscess were densely covered with B. melitensis, group 2, colonies.

Monkey 14 showed that a true B. melitensis, when inoculated subcutaneously, provoked a characteristic remittant temperature reaction. Not every monkey, however, reacted in the same manner, and, judging by the clinical symptoms, monkey 46 would have been considered as refractory. Serologic tests and the demonstration of the inoculated bacteria 94 days after the injection proved the animal to have been infected. Monkey 15 responded to the intravenous injection of a comparatively small dose of a strain of B. melitensis by a prompt and marked production of specific agglutinins. The infection, as judged by the animal's temperature, its general appearance and behavior, was very severe and would have ended fatally. Corresponding to the degree of the infection, the tissues were teeming with B. melitensis.

In our paper dealing with the serologic classification of the Brucella group it was shown that one strain of B. melitensis, our No. 20 (obtained from the Lister Institute, Nov. 1919, marked Austria I, received by them from the Royal Army Medical College, Millbank, London), could not be separated by absorption tests from the group I or B. abortus group. In order to prove that strain No. 20 is really a B. melitensis, the following experiment was carried out:

Exper. 21—Monkey 45.—Pithecus syrichta (Linn.); male, weight 2,625 gm. Fed July 26 and 29, 1920, the light growth of a 48-hour old slant of B. melitensis 20 on carrots, a total of about 70 billion organisms. Under observation for 57 days; slight increase in weight; temperature never above normal. Serum reactions developed as follows:

1st day—agglutination with B. melitensis 20 = 0
10th day—agglutination with B. melitensis 20 = 1:1,000 + + +
15th day—blood cultures sterile
25th day—agglutination with B. melitensis 20 = 1:2,000 + +
B. abortus 80 = 1:600 + + +
57th day—agglutination with B. melitensis 20 = 1:2,000 + + +
B. abortus 80 = 1:4,000 + + +
Chloroformed on Sept. 25, 1920.

Postmortem Examination.—Body in state of excellent nutrition; spleen slightly enlarged and pulp rather soft; other organs normal; no intestinal parasites. Cultures were made from the spleen, liver, kidneys, bile, heart blood, lungs, bone marrow of femurs, mesenteric, inguinal and subscapular lymph nodes. B. melitensis (group 1) was recovered from the spleen and mesenteric lymph nodes.

The prompt appearance of agglutinins in the blood of monkey 45 following the ingestion of two slants of B. melitensis 20 indicates the high invasion properties of this group of bacteria. Moreover, the presumptive evidence is made absolute by the demonstration of the organisms in the spleen and lymph nodes. Cross agglutination and absorption tests proved the isolated strain to be a true melitensis representative of group 1.

It was not considered necessary to repeat experiment 21; the evidence was conclusive that a true melitensis strain differed from a B. abortus strain by its marked invasive properties, when administered by mouth. Two feedings were sufficient to parasitize a monkey, but apparently were unable to produce a characteristic febrile reaction. In the B. abortus experiments, on the contrary, in spite of the fact that a thousand times as many organisms were ingested at each feeding and these repeated over weeks and months, infection only took place when the dosage was tremendous and the bacteria extraordinarily virulent. This can leave no doubt as to the true nature of strain 20, which, while corresponding serologically to the B. abortus group, must be considered a true melitensis organism on account of its marked pathogenicity. This observation is quite in accord with those reported by the British Commission,[15] namely, numerous monkeys in their carefully controlled test series manifested neither febrile reactions nor other clinical manifestations, and yet their blood and organs were literally teeming with B. melitensis. Future studies on the pathogenicity of the representatives of the Brucella group should take these facts into consideration.

One cannot obviously expect the B. abortus to be pyrogenic when entering the tissues via the intestinal tract when the highly pathogenic and invasive B. melitensis quite frequently produces no clinical symptoms. The development of specific agglutinins in the blood serum and the bacterioscopic demonstration of the bacteria in the tissues can be accepted as evidence of true infection.

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[15] Report of Commission, 1907, Part 6, p. 67.

TABLE 1

RESULTS OF FEEDING EXPERIMENTS

Number of Experimental Monkey	Duration of "Feeding" in Days	Number of "Feedings"	Dose of B. abortus at Each Feeding	Method of Administration	Evidence of Infection		
					Presumptive		Absolute
					Appearance of Serum Reaction, Days	Reaction of Serum	B. abortus Recovered From
1	110	1	1 agar slant	Smeared on sliced carrots or bread	—	Negative	Negative
3	38	36	2 agar slants	Smeared on sliced carrots or bread	36th	1:600–800	Spleen
13	22	11	2 agar slants	Smeared on sliced carrots or bread	16th 22d	1:40–80 +++ 1:80–100	Spleen, mesenteric lymph nodes
33	115	64	2 agar slants	Smeared on sliced carrots or bread	42d 123d	1:100 +++ 1:800 ++	Spleen, liver and parasitic abscesses
34	142	82	2 agar slants	Smeared on sliced carrots or bread	—	Negative	No necropsy
43	58	43	1 agar slant (hog strain)	Smeared on sliced carrots or bread	16th 65th	1:80 1:600–1:2000	Spleen and mesenteric lymph nodes
44	58	43	1 agar clant (hog strain)	Smeared on sliced carrots or bread	16th 81st 213th	1:40 1:800 1:80	No necropsy
35	80	52	2 agar slants	Added to 100 c c of pasteurized milk	—	Negative	No necropsy
36	80	52	2 agar slants	Added to 100 c c of pasteurized milk	—	Negative	No necropsy
37	65	44	2/100 agar slant	Added to 100 c c of pasteurized milk	—	Negative	No necropsy
38	65	44	2/100 agar slant	Added to 100 c c of pasteurized milk	—	Negative	No necropsy
19	38	26	Small number	Naturally infected milk	—	Negative	No necropsy
40	53	46	Large number (hog strain)	Naturally infected milk	38th 53d	1:80 1:1000 +++	All organs, heart blood, bile and urine
42	85	52	Small number (hog strain)	Naturally infected milk	—	Negative	Negative

DISCUSSION

The experiments dealing with the intravenous inoculation of B. abortus cultures need no special comment; they definitely indicate that B. abortus is similar in its action on the monkey to B. melitensis. Moreover, the findings serve as a concrete illustration of the prolonged existence of certain bacteria in the tissues of apparently healthy animals.

The infections produced in monkeys by the ingestion of B. abortus cultures, however, require further discussion. For the sake of clearness the results of the feeding experiments are shown in table 1. It has been definitely proven that the oral administration of B. abortus cultures is followed by an invasion of the tissues and the formation of specific agglutinins, provided enormous doses are fed repeatedly for a period covering at least 11 days. Even when observing these provisions, it becomes evident that some monkeys may be refractory. The feeding of milk either artificially or naturally infected produces invasion only in rare instances. This is probably due to the difficulty entailed in feeding the organisms in sufficiently large dosage by this method. These facts stand in absolute contrast to the experiences of the British Commission when working with B. melitensis. In the majority of their experiments, a single oral administration of not more than 5000 B. melitensis was sufficient to infect monkeys. It is apparent that this striking difference in pathogenicity cannot be explained on the assumption that the animals in the experiments with B. abortus received a smaller number of organisms in the milk than those to whom B. melitensis was administered. On the contrary, they ordinarily consumed several thousand times as many abortion bacilli as are necessary to produce a distinct disease when B. melitensis is used. The criticism that the elaboration of specific agglutinins, which was used as presumptive evidence of infection, may not occur on account of the continuous feeding and the corresponding absorption of B. abortus protein, was carefully considered. This cannot alter the conclusions drawn from these feeding experiments because the serologic studies were not made until the monkeys had been under observation a sufficiently long time after the oral administration of antigens had been discontinued, and, when killed, the animals were cultured thoroughly to demonstrate any exciting focus of infection. These feeding experiments, therefore, offer definite evidence that B. abortus is not only serologically different from the predominant groups of B. melitensis, but also distinctly less pathogenic.

It appears profitable for comparison to review briefly in this connection the feeding experiments which have been carried out by several workers on guinea-pigs, rabbits and calves. Smith and Fabyean were the first to observe that suspensions of B. abortus introduced by mouth produce lesions in guinea-pigs. Schroeder and Cotton[16] noted the development of abortion disease in the same species, when feeding them milk of apparently healthy cows that had become chronic carriers of B. abortus. Similar observations have been made in our laboratory. The pathologic lesions produced in guinea-pigs fed small quantities of B. abortus are slight, but positive cultures can practically always be procured from the spleen and lymph nodes of these animals. A positive agglutination reaction in guinea-pigs that have ingested abortion bacilli has always been indicative of an infection, irrespective of the slight tissue changes noted at necropsy. The same results have not been obtained with the rabbit. Experimental production of an infectious disease via the alimentary canal is peculiarly difficult in this type of animal and only prolonged feeding of large numbers of bacteria may lead to an infection (Besredka, Litch and Meyer). Feeding experiments conducted on guinea-pigs and rabbits are of little significance and practical value when used as presumptive evidence of the pathogenicity of an organism for man.

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[16] Amer. Jour. Dis. Child., 1913, 6, p. 340.

Some of the generally recognized facts on the susceptibility of calves to B. abortus feeding infections deserve consideration. Schroeder[17] and Simms and Miller[18] have determined that calves, at least those which are exposed to infection during the first 3 to 5 months of their lives and are known to have ingested an abundance of infected milk prior to weaning, are immune. The two latter observers base their conclusions on the fact that they are not able to demonstrate the development of specific agglutinins in the serum of these calves subsequent to the feeding of the infected milk. According to Huddelson,[19] serum antibodies specific against B. abortus develop occasionally in calves as a result of ingesting naturally infected milk, provided their intestinal tract is made permeable by an existing enteritis. He states, furthermore, that the administration of noninfected milk contaminated with a culture of B. abortus for a period of twelve weeks results in the temporary development of complement-fixing antibodies. The demonstration of fixing substances without the coincidental presence of specific agglutinins appears unusual. From a serologic standpoint these findings are remarkable in view of the fact, that old and recent experimental and clinical observations have shown, that the transmission of active or passive immunity through maternal milk can only take place between homologous individuals of certain groups of mammals in the first few days after birth. This occurrence is, however, by no means frequent and certainly does not follow any definite biologic law. This peculiar behavior of agglutinins is in striking contrast to the positive nursing experiments of Ehrlich and of Römer with antitoxins. The intestinal tract of young animals can probably absorb agglutinins derived from and present in the milk of the same species more readily than those obtained from heterologous ones. Moreover, the review of Römer[20] on this subject emphasizes the fact that instances of definitely proven transmission of human typhoid agglutinins through the milk of nursing mothers to their infants are not available. These observations are inconsistent with Cooledge's conclusions relative to the absorption of heterologous agglutinins through the intestinal tract of man. If these negative feeding experiments of Schroeder, Simms and Miller on calves are confirmed by other observers, it must be concluded that the intestinal mucosa of these animals is not permeable to homologous maternal milk agglutinins nor for the specific abortion bacilli.

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[17] Report U. S. Live Stock Assn., 1919, p. 125.

[18] Jour. Amer. Vet. Med. Assn., 1921, 58, p. 533.

[19] Michigan Agric. Exper. Station Bull., No. 32, 1916.

From an epidemiologic standpoint the failure of calves to develop specific agglutinins following the feeding of infected milk is of tremendous interest, if one appreciates that adult bovine animals must be considered exceptionally susceptible to B. abortus and that infections are believed to result from the ingestion of this organism. It is obvious that important factors predisposing to a B. abortus feeding infection are as yet unknown, but it appears probable that the actual number of ingested bacteria plays an important rôle. The successful feeding experiments of Bang, of MacFadyean and Stockmann and of Zwick and Zeller on adult pregnant cows and goats have been carried out by feeding emulsified placentas or very heavy cultures. In several tests of Zwick and Zeller[21] the oral administration of cultures led rapidly to the development of serum agglutinins; in only one instance, however, to abortion and the demonstration of B. abortus in the tissues of the experimental goats and sheep. Similar observations have been made with B. melitensis by the British Commission. The feeding of goats with infected milk or cultures was invariably followed by the production of specific agglutinins, but the infection could only be confirmed at necropsy in 40% of the experiments. These observations support the contention that the development of serum agglutinins in animals following the oral administration of B. abortus or B. melitensis either in culture or in milk is indicative of an infection and that B. melitensis is more invasive to ruminants, therefore effective in smaller dosage.

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[20] Sommerfeld, Handbuch. d. Milchkunde, 1909, p. 486.

[21] Arb. a. d. k. Gsundhtsamte, 1912, 43, p. 44.

Reference to the dosage necessary for a B. abortus infection naturally leads to a discussion of the "virulence" of these bacteria. It must be admitted that our knowledge on this phase of the problem is most incomplete. Guinea-pigs, the usual test animals, exhibit a variable susceptibility, and carefully controlled quantitative experiments have apparently not been carried out. In addition, there is a tremendous degree of variability in the pathogenicity of the different strains. For example, the so-called "hog strain" of B. abortus employed in our experiments was highly pathogenic. Guinea-pigs inoculated with 2 to 5 million organisms succumbed regularly to a generalized B. abortus infection in from 3 to 4 weeks. The necropsy findings revealed only slight emaciation, a spleen tumor and cloudy swelling of the parenchymatous organs. In the test tube the strains grew readily and abundantly. In comparing a large number of strains and their pathogenicity for guinea-pigs, the impression is gained that growth vigor in the test tubes as a rule runs parallel with a high degree of pathogenicity not only for guinea-pigs, but also for other animals. Opportunity will be afforded to discuss this phase of the problem in a paper dealing with the pathogenicity of B. abortus and B. melitensis for laboratory animals. In the feeding experiments on monkeys these differences in virulence were decidedly in evidence. In the light of these findings it is justifiable to anticipate that future bacteriologic work may reveal a strain of B. abortus, which, in relation to its invasive properties, approaches the B. melitensis group more closely than the strains thus far studied.

These possibilities immediately suggest the important question, "Is B. abortus pathogenic for man?" In previous papers the literature relative to this problem has been reviewed and certain data presented in which the most striking evidence as to pathogenicity for man rests on the following facts: (a) Mohler and Traum,[22] by inoculating guinea-pigs, found B. abortus present in one out of 56 tonsils obtained from children; (b) Larson and Sedgwick,[10] Nicholl and Pratt,[11] and Cooledge [13] demonstrated "specific agglutinins and fixing antibodies in the blood of a small percentage of children and adults, who consumed raw milk; (c) B. abortus belongs to the Brucella group and is closely related to B. melitensis, which is highly pathogenic to man.

The experiments of Mohler and Traum have been repeated by Nicholl and Pratt, with negative results, but aside from this the literature is barren of careful bacteriologic studies of those children's diseases in which B. abortus might have been suspected as an etiologic agent. The aforementioned indirect isolation of B. abortus from the tonsils must therefore be considered the only instance in which this organism has been isolated from human tissues. The value of the finding is seriously invalidated by the fact that spontaneous B. abortus infections occasionally occur in laboratory animals accidentally exposed. Until provision can be made to prevent outside spontaneous infection, a repetition for confirmation of the studies of Mohler and Traum is not feasible.

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[22] Twenty-eighth Ann. Report Bur. Animal Ind., Dept. of Agric., 1911, p. 157.

The demonstration of certain anti-bodies in the blood serum of children and adults deserves further experimental investigation. In view of our negative results in a limited series of about 50 serums obtained from children who consumed raw, certified milk, known to contain B. abortus, the positive findings hitherto reported can scarcely be considered constant. The bacteria of the Brucella group are quite readily clumped nonspecifically. This subject has been well covered in several papers on B. melitensis agglutination by Euzière and Roger, Rouslacroix,[23] Nègre and Raynaud[24] and others. In addition, the occurrence of "inhibition zones" is quite common and the serologist must distinguish between coarse and fine clumping of the bacterial emulsion. Only when these limitations have been carefully noted and the nonspecific agglutinins destroyed by heating to 50 C., as recommended by Nègre and Raynaud, can the reaction be considered specific. The frequent errors in the diagnosis of undulant fever reported in several publications demonstrate that only an agglutination in a dilution above 1:100 is indicative of a true infection. We have not as yet tested a sufficiently large series of human serums by means of the complement-fixation reaction to draw conclusions. The negative findings of Williams and Kolmer[25] on 50 serums demonstrate that even when one's technic is correct suggestive reactions are exceedingly rare. The observations reported in the literature, which have been previously discussed, in addition to the feeding experiments on cattle, guinea-pigs, rabbits and monkeys, definitely demonstrate that heterologous antibodies in cow's milk are probably never absorbed in the large intestines of adult human beings. The solution of the problem of human pathogenicity can probably not be reached by serologic studies alone but must depend on extensive tissue cultures.

Considering the close relationship between B. abortus and B. melitensis, it seems remarkable that a B. abortus disease resembling undulant fever has never been recognized in countries in which abortion disease in cattle is common. Several explanations can be offered. B. abortus is unquestionably less pathogenic and invasive than B. melitensis. It is a well-known fact that great care must be exercised in handling cultures of the B. melitensis as bacteriologists working with the organism have become infected in an unusually high proportion of instances as compared with other affections. The laboratory infections reported by Carbone,[26] by Birt and Lamb[27] and by Strong and Musgrave[28] are in striking contrast to the absence of instances dealing with B. abortus infections either among laboratory workers, farmers or veterinarians, who unquestionably come in most intimate contact with large numbers of virulent abortion bacilli. Alice C. Evans points out that the number of virulent B. abortus, which persist in the milk of cows is not great, probably even so small as to be negligible from the standpoint of food infection. On the other hand, the milk of malta fever goats is usually teeming with bacteria, in some instances more than 1 million per c c. It is recalled that goats artificially infected with abortion bacilli may discharge enormous numbers of bacteria in their milk, while according to Shaw[29] cows naturally infected with B. melitensis (?) may eliminate comparatively few bacteria. The sediment of 10 to 15 c c milk, which had been centrifugalized, gave for one cow on 7 occasions 3-40 colonies, and from another 7-231 colonies. These observations either indicate that the number of B. melitensis in cow's milk is relatively small or, what is more likely to be true, that the bacteria demonstrated by Shaw were abortion bacteria, which he could not differentiate with his agglutinating serum from B. melitensis. It is probably, however, not so much the amount of virus necessary to cause an infection as the low pathogenicity of the organism for man which explains our freedom from a disease definitely proved to be due to B. abortus.

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[23] Gaz. d. hôp., 1912, 85, p. 289.

[24] Compt. rend. Soc. de biol., 1912, 72, p. 664.

[25] Amer. Jour. Obst., 1917, 75, p. 193.

In the paper on B. abortus in certified milk it was pointed out that the number of abortus bacilli present in this food was small and the excretion probably variable to a large degree. The assumption that abortion bacilli cannot be demonstrated in cow's milk by direct plating has been refuted by the observations of Huddelson and also of Steck, who isolated the bacteria on ordinary dilution plates. Until our knowledge relative to the actual number of B. abortus organisms in

[26] Arch. per le sc. med. Torino, 1904, 28, p. 273.

[27] Lancet, 1899, 2, p. 701.

[28] Philadelphia Med. Jour., 1900, 6, p. 996.

[29] Report of Commission, 1906, Part 4, p. 23.

market or certified milk is based on similar detailed tests as reported for milk of malta fever goats, it would be unwise to explain our apparent freedom from "abortion disease" by the low number of viable and invasive bacteria. A sudden increase in virulence as shown in one of our goats may lead to a discharge of enormous numbers of abortion bacilli from the udder.

It is impossible to predict and to estimtae the consequences of such an event on the human body. An answer to the question—"Is B. abortus pathogenic for man?"—must therefore be kept sub-judice even if the evidence thus far collected and critically analyzed encourages the belief that B. abortus is under ordinary conditions nonpathogenic.

CONCLUSIONS

Following an intravenous inoculation of B. abortus, agglutinins develop fairly rapidly in the blood of monkeys. The animals may show an intermittent type of fever and lose weight. At postmortem examination it is possible to recover the organisms from the spleen, lymph nodes and liver, while even on the fourth day after the injection the blood stream is found sterile.

By feeding the growth of glycerol agar cultures of moderately virulent strains of B. abortus on carrots, bread or apples in large and repeated doses, it is possible to obtain definite infections in a fairly large percentage of monkeys. The duration of incubation as judged by the appearance of serum agglutinins is always prolonged and at least 30 to 40 days may elapse until such substances can be demonstrated. The infection can be positively proven by the isolation of B. abortus from the spleen and occasionally from other tissues.

The repeated oral administration of massive doses of highly virulent B. abortus cultures causes a rapid development of specific agglutinins; by the 16th day the serum may be active in a dilution of 1:40-80. Interruption of the feeding leads to a gradual diminution of the antibodies, probably on account of the disappearance of the bacteria from the tissues.

Milk of cows heavily contaminated with cultures of B. abortus of low virulence when fed for at least 52 days failed to cause infections. The mere ingestion of cow's milk rich in agglutinins never led to an appearance of these substances in the serum of monkeys.

Milk obtained from goats, which had been experimentally infected with B. abortus of low virulence and which contained relatively few bacteria, failed to produce an infection in monkeys even when fed for 12 to 42 days.

By feeding repeatedly the milk of a goat that had been infected by the injection into the udder of a very virulent strain of B. abortus of porcine origin, it was possible to infect one monkey. B. abortus was isolated at necropsy from all the viscera and the heart blood.

Serum agglutinins specific for the bacteria of the Brucella group are formed only in the presence of a definite infection. The ingestion of heat-killed, autolyzed abortion bacilli is antigenically ineffective in monkeys.

Certain strains of B. melitensis, which produce characteristic lesions in guinea-pigs resembling those of B. abortus, when injected into monkeys intravenously give rise to characteristic temperature, positive agglutination reactions, and the organisms are recoverable at the post-mortem examination from various tissues.

One atypical melitensis strain introduced via the alimentary canal was not always pyrogenic, but it stimulated agglutinins and the ingested bacteria persisted for at least 57 days in the spleen and mesenteric lymph nodes.

The microscopic changes produced by a B. melitensis or by a severe B. abortus infection in the monkey resemble those of an early typhoid infection.

SUMMARY

Virulent strains of B. abortus in sufficiently large dosage are pathogenic for monkeys.

B. melitensis is far more invasive than B. abortus. One or two feedings of one-thousandth the amount necessary to cause an infection with B. abortus is sufficient in melitensis infection to parasitize a monkey.

APPENDIX

HISTORIES OF GOATS WHICH FURNISHED INFECTED MILK FED TO THE MONKEYS

GOAT 1

June 17-20,1919: About 820 c c of milk were collected daily. (3rd month of lactation); Trommsdorff's test, 0.03%; cultures made with the centrifugalized sediment remained sterile; blood serum and whey agglutination tests with B. abortus and B. melitensis were negative.

June 21: Infected by the injection of the growth of a 24-hour old agar slant of B. abortus 80 into the right teat canal. The inoculated half of the udder was not milked until June 28; the daily temperatures of the goat remained normal.

June 28: The right udder was tender, the posterior portion was hard and nodular; 60 c c of milk were collected from the right and 350 c c from the left udder. The milk of the right udder was colostrum like, rich in leukocytes and contained 80-100,000 B. abortus per c c.

June 29—July 7: Daily 100 c c of milk from the right udder; Trommsdorff, 0.05%; the appearance of the milk was normal; slant cultures showed ∞ B. abortus colonies.

July 8: The blood serum of the goat agglutinated B. abortus 80 in a dilution of 1 : 1,000; the udder was still nodular and tender; 1 c c of milk from the right udder contained approximately 20,000 abortion bacilli; the milk of the left udder was sterile.

July 17: Collected 100 c c of milk from the right udder; 1 c c of the milk contained 50 viable B. abortus; Trommsdorff, 0.02%.

July 18—Aug. 28: Collected daily 110-150 c c of milk from the right udder.

Aug. 13, 15, 21 and 28: Milk sample cultures for B. abortus remained sterile. Milk collection from Aug. 12—Sept. 13 inclusive fed to 4 guinea-pigs; failed to provoke abortion disease in these animals.

Sept. 1-26: Collected daily an average amount of 126 c c of milk from the right, and an average of 238 c c from the left udder; the whey agglutinated B. abortus in a dilution of 1 :80—1 :100 + + +.

Sept. 27: Infected the left udder by inoculating the growth of one slant each of B. abortus 80 and 14 through the teat canal.

Sept. 28: The left udder was enlarged, hot and tender; the goat registered a rectal temperature of 40.4 C. and refused to eat.

Sept. 29: The temperature of the goat was normal; the secretion of the left udder, about 5 c c, consisted of curdled milk, very rich in leukocytes; B. abortus was demonstrated by smears and cultures; the right half of the udder secreted a sterile milk.

Sept. 30-October 10: Collected 5-30 c c of yellowish milk from the left and 100-170 c c of milk from the right udder. Agglutination tests of whey were positive in dilutions of 1 :100—200 + + +.

Oct. 12: Secretion from the left udder less than 1 c c.

Oct. 20. The goat was bred; secretion from the right udder 50 c c.

Oct. 21-Nov. 10: Milk secretion from the right udder declined from 60 c c to 15 c c and on Nov. 11 there was no longer any secretion from the udder.

March 13, 1920: The goat gave birth to 3 dead but full grown kids; organs of the fetuses were found to be free from B. abortus; milk secretion from both udders was excellent.

March 14 and 15: The goat had retentio placentarum; the blood serum agglutinated B. abortus 80 in a dilution of 1 :40; the milk whey of the right and left udders was positive, in a dilution of 1 :10; the cultures made from the centrifugalized milk sediment were sterile.

March 25: Infected right udder by inoculating into the teat canal the growth of one slant of B. abortus ("hog's liver"), about 85 billion organisms.

March 26-April 1: The udder was very tender and edematous; the supramammary lymph nodes were enlarged and firm. The goat had a toxic appearance, ate little, failed to ruminate and lay in a stupor in the corner of the pen. The coat was rough and the temperature rose daily, in the evening above 40.8 C. The secretion collected from the right udder (not more than 50 c c) was blood-tinged, partially curdled, rich in leukocytes and teeming with B. abortus.

April 2-8: The goat improved clinically; the secretion varied between 35-70 c c from the right and 200-310 c c from the left udder. The temperature of the goat was normal.

April 12: The blood serum of the goat agglutinated B. abortus 80, 1 :2000; the milk whey of the left udder was positive in a dilution of 1 :800 and the one of the right udder in a dilution of 1 :2000 + + +.

April 13-August 28: The goat appeared well, gained in weight and secreted between 60-125 c c milk from the right and 200-300 c c from the left udder. The right half exhibited on palpation numerous nodular indurations; similar but less marked areas were noted in the left udder. Both supramammary lymph nodes were the size of large eggs, very hard and fibrous, but not tender; the leukocyte count of the physically normal milk varied between 6-15,000,000 per c c of secretion obtained from the right and the left udder. The agglutination reactions with serum and milk whey are shown in table 2.

TABLE 2

AGGLUTINATION REACTION WITH SERUM AND MILK WHEY

Date	Day after Third Infection	Serum Value	Milk Whey Agglutinates	
			Left Udder	Right Udder
March 25, 1920..................	0	1:40	1:10	1:10
April 12, 1920..................	18	1:2000	1:800	1:2000
April 23, 1920..................	29	......	1:400	1:400
May 10, 1920..................	46	......	1:1000	1:600
May 22, 1920..................	58	1:1000	......	......
June 23, 1920..................	90	......	1:100	1:600
July 31, 1920..................	128	1:2000	1:1000	1:40

Oct. 25: There was no longer any secretion from the udder. The goat was bred.

March 10: The goat gave birth to 3 mature, healthy kids; the milk collected on March 14, 16, 18 and 20 was sterile; the blood serum aggluinated B. abortus in a dilution of 1:20; the milk whey was negative in a dilution of 1:20.

TABLE 3

NUMBER OF B. ABORTUS ISOLATED FROM 1 C C OF THE SECRETION OF EACH UDDER

Date	Day of Third Infection	Number of B. abortus per c c of Milk	
		Left Udder	Right Udder
March 25, 1920............	0	Sterile	Sterile
March 27, 1920............	2	Sterile	Dilution too low
April 8, 1920............	14	50	40,000
April 23, 1920............	29	1,500	250,000
May 10, 1920............	46	180,000	30,000
June 1, 1920............	68	11,800	4,000
June 25, 1920............	93	0	20
July 24, 1920............	121	0	10 per loop of sediment
Aug. 19, 1920............	147	0	3-5 per loop of sediment
Aug. 31, 1920............	159	0	0

GOAT 2

May 27, 1920: Milk whey from both udders gave negative agglutination reactions; blood serum unheated agglutinated B. abortus in a dilution 1:40 + + +; heated serum was negative.

June 30: Infected by direct inoculation into the right teat canal of 5 c c representing the growth of one agar slant each of B. abortus 60, 100 and 11 (all recently isolated and obtained from Dr. Ward Giltner, East Lansing, Mich.).

July 1-8: The goat was perfectly well; the udder was slightly tender and edematous, but the right udder was not milked.

July 9-18: Daily 15-25 c c of milk were collected from the right and 50-70 c c from the left udder.

July 19: Milk directly plated was sterile; the sediment of 15 c c of milk of each udder gave the following number of colonies; right about 48, left 10 colonies per loopful.

July 24: A similar test gave the following result: right 4, left 12 colonies per loopful of sediment.

July 28: The blood serum agglutinated B. abortus 80 in a dilution of 1:2000 + + +; the milk whey of the left udder—1:80 + + +; the milk whey of the right udder—1:2000 + + +.

July 29-Aug. 31: The daily collection of milk gradually dropped from 150 c c to 35 c c. Repeatedly tested, the sediment was found to be sterile, and when inoculated into guinea-pigs failed to provoke abortion disease.

Sept. 10: There was no longer any secretion of milk. Blood serum agglutinated B. abortus in a dilution of 1:800 + + +.